Influence of the Homogenisation Procedure on the Physicochemical Properties of PLGA Nanoparticles

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Pilocarpine HCl-loaded PLGA nanoparticles were prepared by emulsification solvent evaporation. Three different stabilisers, polyvinylalcohol (PVA), Carbopol and Poloxamer were used, as well as mixtures thereof. The influence of the homogenisation pressure and number of cycles on the properties of nanoparticles were studied. Particle size was shown to depend on the stabiliser used. An increase of the homogenisation pressure or the number of cycles resulted in a decrease in particle size. The zeta potential value was influenced mainly by the nature of the stabiliser. Particles stabilised with poloxamer or PVA showed a slightly negative zeta potential value, while samples stabilised with carbopol posessed a more negative zeta potential, which became less negative after homogenisation. Drug encapsulation depended strongly on the stabiliser used. The higher drug entrapment of the carbopol-stabilised particles could be explained by an electrostatic interaction between the negatively charged carboxyl groups of carbopol and the positively charged, protonated pilocarpine. The drug release patterns of the particles prepared were quite similar. Differences between the release patterns of the homogenised particles could be attributed both to differences in size as well as drug encapsulation. Turbidimetric measurements suggested an interaction between mucin and PLGA nanoparticles exclusively stabilised with Carbopol.

Key words PLGA; nanoparticle; homogenisation; mucoadhesion

One of the most popular techniques for the preparation of PLGA nanoparticles is emulsification solvent evaporation and its modifications such as the use of a double emulsion for the encapsulation of water soluble drugs.[1,2]

In the current study, a homogenisation step was added to the preparation procedure. The effect of the stirring speed used during emulsification on the PLGA particle properties has been extensively examined.[3,4] However, high stirring speed and even sonication are often not sufficient to achieve a small particle size and/or a narrow particle size distribution. Therefore, the high-pressure homogenisation technique was successfully adapted.[5] This method is mainly used for the production of microemulsions[6—8] and liposomes,[9,10] but there is only a limited number of studies concerning polymeric nanoparticle preparations.[11—13]

The particle size is, however, an important parameter co-determining the particle distribution and drug release. More specifically for ophthalmic use, it has been observed that large particles may irritate the eye. Consequently, smaller particles are preferred for ophthalmic delivery systems.[14] Additionally, Calvo *et al.* have reported that poly(ε-caprolactone) nanoparticles (0.20—0.25 μm) improve ocular bioavailibility of indomethacin rather than poly(ε-caprolactone) microparticles (6 μm).[15] Thus, one of the most important characteristics of nanoparticles is their size.

The present study aimed to evaluate the feasibility of homogenisation as a tool to adapt the properties of pilocarpine HCl-loaded PLGA nanoparticles. The influence of the homogenisation procedure on the size, the zeta potential value, drug encapsulation and drug release of the particles prepared was studied.

The homogenisation parameters studied were the pressure applied, as well as the number of cycles.

PLGA particles were prepared using three different stabilisers: polyvinylalcohol (PVA), carbomer (Carbopol 980 NF) and poloxamer (Lutrol F-68). PVA is the reference stabilising agent for the emulsification-solvent evaporation method.[3] The feasibility of the use of Carbopol and poloxamer has been demonstrated in earlier work.[16] Apart from these three stabilisers, mixtures of PVA and poloxamer as well as mixtures of PVA and Carbopol were also employed.

The ocular bioavailibility can be improved using mucoadhesive particulate drug delivery systems.[17] Various methods have been developed to evaluate the mucoadhesion of nanoparticulate drug delivery systems. Determination of the detachment force for microspheres from pig intestinal mucosa[18] or from mucus model gel[19] was proposed. Another method was to evaluate the amount of attached or non-adherent nanoparticles after placing on the rat intestinal mucosa.[20] Some authors investigated the behaviour of mucin/bioadhesive polymer dispersions by rheology[21,22] or by turbidimetric measurements.[23] Thus, the interaction between mucin and mucoadhesive products caused changes in the viscosity or turbidity of the dispersions. Since these techniques are easily accessible it would be interesting to adapt their use for nanoparticle systems. In the present study, preliminary turbidimetric data is presented on the interaction between mucin and PLGA nanoparticles stabilised by PVA, Carbopol and a mixture of both.

Experimental

Materials Pilocarpine hydrochloride was purchased from Federa (Brussels, Belgium). Poly(DL-lactide-co-glycolide) (PLGA, Resomer RG 503, lactic : glycolic ratio 52 : 48, MW 40000) was obtained from Boehringer Ingelheim (Ingelheim, Germany). Polyvinylalcohol (PVA) (Average MW 30000—70000) and gastric mucin (type II: crude) were supplied by Sigma Chemical Co. (St. Louis, U.S.A.). Methanol and acetonitrile (HPLC grade) were from Acros Organics (New Jersey, U.S.A.) and methylene chloride from Aldrich (Gillingham, U.K.). Carbopol 980 NF was obtained from BF Goodrich (Cleveland, U.S.A.) and Poloxamer 188 (Lutrol F-68) was from BASF (Ludwigshafen, Germany). NaCl was delivered by Merck Eurolab (Leuven, Belgium) and KCl, $NaHCO_3$, $CaCl_2 \cdot H_2O$ and $MgCl_2 \cdot 2H_2O$ were from Merck (Darmstadt, Germany).

Methods. Particle Preparation The PLGA nanoparticles were produced using a w/o/w emulsification solvent evaporation method. An amount

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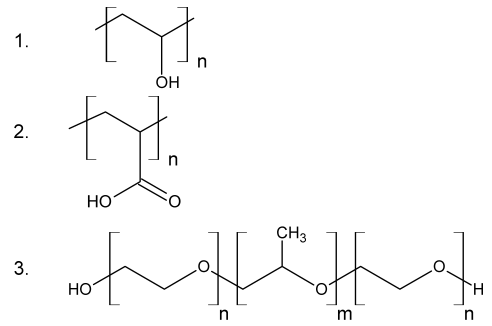

1. PVA 2. Carbopol 3. Poloxamer

Fig. 1. Chemical Strucutres of the Stabilisers Employed to Stabilise PLGA Nanoparticles

Table 1. Concentrations of the Stabilisers Used for the Preparation of PLGA Nanoparticles

	Stabiliser concentrations (% w/v)	
	First outer water phase	Second outer water phase
Stabilisers used as such		
PVA	1.000	0.330
Carbopol	0.012	0.004
Poloxamer	2.200	0.730
PVA and Carbopol mixture		
PVA	0.500	0.165
Carbopol	0.006	0.002
PVA and Poloxamer mixture		
PVA	0.006	0.002
Poloxamer	1.100	0.365

of 1.00 g of PLGA polymer was dissolved in 10 ml of methylene chloride. A volume of 2.0 ml of an aqueous pilocarpine HCl solution (2.5% w/v) was added to the organic phase containing the polymer, after which the mixture was sonicated for 1 min at 80 W (Branson Sonifier B-12, Danbury, U.S.A.) to form a w/o emulsion. This emulsion was subsequently added to 50.0 ml of an outer water phase containing the stabilisers to be tested: PVA, Carbopol or poloxamer or a mixture of two of those. The structures of these stabilisers are shown in Fig. 1. Again, sonication was applied for 30 s leading to formation of a w/o/w emulsion. This emulsion was then homogenized (M-110L, Microfluidics, Newton, U.S.A.), to reduce the emulsion droplet size and to obtain a more narrow size distribution. The homogenisation pressures applied were 100 and 500 bar, respectively, and the number of cycles was one and three. As a reference, spheres were prepared without homogenisation. The homogenised solutions were then poured into 150 ml of a second outer water phase containing a stabiliser or stabiliser mixture and were stirred at 700 rpm (Variomag Poly 15, Munchen, Germany) to allow evaporation of the organic phase, resulting in precipitation of the PLGA polymer as nanoparticles. The suspensions were cooled down at −18 °C and then freeze dried. The concentrations of the stabilisers employed are summarised in Table 1.

Particle Evaluation. Particle Size Particle size was determined by photon correlation spectroscopy with a Zetasizer 3000 (Malvern Instruments, Malvern, U.K.). Samples of the freeze dried preparations were redispersed in distilled water under magnetic stirring before measuring. Each sample was determined four times and average values were calculated.

Particle Zeta Potential The particle zeta potential values were measured by laser doppler anemometry using a Zetasizer 3000 (Malvern Instruments, Malvern, U.K.). In order to determine the electrical charge of the particles produced after instillation in the tear film, the zeta potential was measured in Simulated Lachrymal Fluid (SLF), an isotonic electrolyte solution, resembling the composition of the tear film.[24)]

Samples of the freeze-dried preparations were redispersed in SLF under magnetic stirring before measuring. Each sample was measured 10 times and the average values were calculated.

Table 2. Particle Sizes±S.D. (nm) of the Pilocarpine HCl-Loaded PLGA Nanoparticles Prepared Using PVA, Poloxamer or Carbopol as Stabiliser

	Homogenisation pressure (bar); number of cycles				
	0	100 ; 1	100 ; 3	500 ; 1	500 ; 3
PVA	333±6	283±3	232±9	231±5	204±4
PVA+Pol	512±3	294±5	260±6	255±4	221±8
PVA+Carb	408±8	300±4	246±3	278±5	205±5
Poloxamer	572±4	691±7	425±4	467±9	304±5
Carbopol	1125±76	631±9	366±8	467±12	309±6

Drug Loading Freeze dried nanoparticles (20 mg), accurately weighed, were dispersed in 10.00 ml MilliQ water by sonication for 10 min. The samples were centrifuged at 3000 rpm for 3 h and the drug content in the supernatant was determined by a validated HPLC method. The HPLC system was a Gilson 321 pump (Villiers-le-Bel, France). The mobile phase consisted of a water/methanol mixture (97 : 3, v/v) and potassium dihydrogen phosphate (5%, w/v). Determinations were performed using a column μBondapak C_{18} 125 Å 10 μm (Waters) at a flow rate 2 ml/min and sensitivity 0.005%, respectively. Pilocarpine hydrochloride was detected at 216 nm and its concentration was calculated according to the calibration curve prepared under the same conditions. The measurements were made in duplicate. The encapsulation efficiency (EE) for all samples was estimated using the equation:

$$\text{encapsulation efficiency (\%)} = \frac{\text{actual drug loading}}{\text{theoretical drug loading}} 100\%$$

Drug Release The *in vitro* release studies were carried out in duplicate using diffusion cells at room temperature. The acceptor and donor compartments of the horizontal cells were separated by a dialysis membrane (Mw cut off 12000—14000 D, Medicell, U.K.). The nanoparticles (20 mg) were placed as aqueous suspensions (2.0 ml) in the donor compartments of the cells. The acceptor compartments were filled with 18 ml distilled water and stirred magnetically at 200 rpm. At suitable time intervals aliquots of 0.8 ml were withdrawn from the acceptor compartments and replaced by the same volumes of fresh distilled water. The concentrations of samples were determined by the above described HPLC method.

Turbidimetric Measurements Turbidimetric measurements were performed by means of a Hitachi U-1500 spectrophotometer at 650 nm. The accurately weighed nanoparticles were added to the 0.1% mucin dispersion (w/w) in water or 0.25% mucin (w/w) in SLF and stirred at 200 rpm. Two different concentrations (0.5, 1 mg/ml) of nanoparticles were examined in a 1 : 2 or 1 : 1 nanoparticle : mucin ratio (w/w). The turbidity of the dispersions was measured at certain time intervals and compared to the turbidity of the native mucin dispersion. The behaviour of nanoparticles was also examined in a mucin free dispersion under the same conditions. All measurements were performed in triplicate.

Results and Discussion

Particle Size The results of the particle size measurements are presented in Table 2. Particle sizes ranging from 330 to 1100 nm were obtained when no homogenisation was applied, indicating that the stabiliser used has an influence on the resulting particle size. The smallest particles were obtained when PVA was used, so one could state that PVA is more efficient in stabilising the emulsion formed during particle preparation than the other two stabilisers tested. The results of the particles obtained with stabiliser mixtures also show the strong stabilising effect of PVA. As PVA was mixed with carbopol or poloxamer, particle sizes were obtained which were usually a little higher than those measured when PVA was used as such, indicating that the stabilisation of the emulsion during particle formation largely depended on the presence of PVA and much less on poloxamer or carbopol.

When the results of the homogenised particles are compared to those of the non-homogenised ones, a reduction in

Table 3. Zeta Potential Values±S.D. (mV) of the Pilocarpine HCl-Loaded PLGA Nanoparticles Prepared Using PVA, Poloxamer or Carbopol as Stabiliser

	Homogenisation pressure (bar); number of cycles				
	0	100 ; 1	100 ; 3	500 ; 1	500 ; 3
PVA	−20.2±2.2	−13.3±5.6	−11.6±4.1	−13.8±1.4	−16.1±3.8
PVA+Pol	−27.2±1.0	−13.6±3.8	−10.4±6.1	−18.8±1.6	−13.7±1.2
PVA+Carb	−19.0±2.8	−18.4±2.2	−11.6±2.7	−17.2±1.7	−17.5±3.6
Poloxamer	−14.1±5.6	−12.7±5.1	−8.7±4.2	−10.5±1.1	−11.7±4.9
Carbopol	−42.2±2.0	−32.6±4.1	−22.9±6.1	−40.1±5.5	−19.7±5.6

particle size is observed. This effect was stronger for carbopol and poloxamer. For particles prepared with PVA the same effect occured, but the absolute reduction of the particle size is in this case smaller, as very small particles were already produced without the application of homogenisation, due to the good stabilising properties of PVA.

There is a clear trend of size reduction when the homogenisation pressure and number of cycles are increased. This phenomenon can be explained by the formation of smaller emulsion droplets during homogenisation at higher pressures or numbers of cycles, eventually resulting in the formation of smaller nanoparticles.

Zeta Potential Value The results of the zeta potential value measurements are presented in Table 3. The zeta potential values for particles prepared with PVA and poloxamer are slightly negative, around −10 to −20 mV. Particles stabilised with carbopol, however, have a more negative zeta potential. The first conclusion that can be drawn from the data obtained is that the stabiliser employed co-determines the zeta potential. The difference in zeta potential values for different stabilisers indicate that they are present at the particle surface, or at least influence the electrical charge distribution at the particle surface. The more negative values obtained for carbopol-stabilised nanoparticles can be explained by the fact that carbopol contains carboxyl groups which can be deprotonated, leading to a negatively charged polymer chain. If the carbopol molecules are present at the particle surface, their negative charges could contribute to a more negative zeta potential value. PVA and poloxamer, on the other hand, carry no electrical charges, resulting in a less negative value of the zeta potential.

When the data of the particles stabilised with the mixtures is considered, similar values for the mixture poloxamer-PVA were measured compared to the results obtained with PVA and poloxamer alone. However, when the mixture PVA-carbopol was used, zeta potential values almost identical to those obtained with PVA alone were observed. One could expect values between those obtained with carbopol and PVA as single stabilisers, but, as was the case with particle size, the effect of PVA on the zeta potential seems to be much stronger than the effect of carbopol. One could assume that carbopol and PVA are both present at the particle surface when the mixture was employed, but that the presence of PVA almost totally 'masked' the presence of the negative carboxyl groups of carbopol.

The influence of the homogenisation pressure and number of cycles is less clear. For PVA and poloxamer, the differences observed are rather small. In the case of carbopol, however, a decrease of the zeta potential value is observed as the homogenisation pressure or number of cycles is raised. During the homogenisation, the size of the emulsion droplets, which will ultimately determine the size of the nanoparticles, is reduced. As higher pressures or more cycles are applied, the emulsion droplet size further drops. This results in smaller particle sizes, and consequently in less stabiliser available per surface unit to cover the particle. Since less negatively charged groups from carbopol are present per surface area unit, the zeta potential value of the particles becomes less negative. The correlation between particle size and zeta potential can also be deduced from Fig. 2, in which the particle size and the corresponding zeta potential values of the particles prepared are presented. For the sake of clarity, only the data points for the particles prepared using only one stabiliser are presented. The straight line in Fig. 2 is fit through the data points and seems to suggest a more negative zeta potential for larger particles (correlation coefficient −0.6035). This trend is mainly explained by the difference between the larger particles produced with carbopol as a stabiliser, having a more negative zeta potential value on one hand, and the smaller particles stabilised with either PVA or poloxamer, having a smaller zeta potential value on the other hand. When the preparations are considered per stabiliser, the correlation between particle size and zeta potential is most clear for carbopol.

Drug Loading The results of the drug loading determinations are presented in Table 4.

When particles were prepared without homogenisation drug encapsulations between 20 and 70% were obtained, with the lowest value for particles stabilised with a PVA-poloxamer mixture and the highest for Carbopol. Consequently, the drug encapsulation depends on the type of stabiliser. When homogenisation was applied during particle preparation, the drug encapsulation is reduced especially when the homogenisation pressure or the number of cycles is increased. When carbopol was employed, however, encapsulation remained high and only dropped to 32% at a combination of 500 bar and 3 cycles. The data of the particles prepared with stabiliser mixtures reveal the same trends: a higher drug encapsulation when no homogenisation is applied, and a decrease when the pressure or the number of homogenisation cycles is increased.

The lower drug encapsulation at higher homogenisation pressures or number of cycles is probably due to the enhanced diffusion of the hydrophilic pilocarpine hydrochloride molecules out of the emulsion droplets during their size reduction under pressure. Similar results were reported by Soriano *et al.*[25] They found that albumin-loaded PLGA microspheres manufactured under pressure had a lower encapsulation efficiency than the samples produced only by sonication.

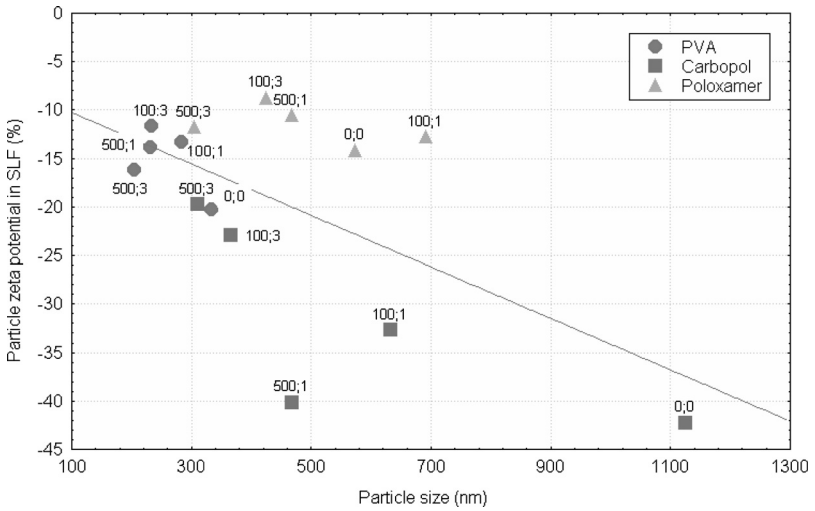

Fig. 2. Correlation between Particle Size and Zeta Potential of Pilocarpine HCl-Loaded PLGA Nanoparticles Prepared with PVA, Carbopol or Poloxamer as Stabiliser

At each data point the pressure applied during homogenisation and the number of cycles are printed.

Table 4. Drug Encapsulation±S.D. (%) of the Pilocarpine HCl-Loaded PLGA Nanoparticles Prepared Using PVA, Poloxamer or Carbopol as Stabiliser

	Homogenisation pressure (bar); number of cycles				
	0	100 ; 1	100 ; 3	500 ; 1	500 ; 3
PVA	61.5±12.4	16.6±5.3	13.9±3.9	4.4±1.0	20.0±8.2
PVA+Pol	16.4±6.3	18.5±3.2	10.1±4.1	10.1±3.7	7.6±4.1
PVA+Carb	41.8±12.1	21.0±14.6	12.8±7.2	28.4±9.6	20.8±8.4
Poloxamer	32.6±6.2	30.0±4.6	16.0±3.6	27.9±9.8	16.8±5.6
Carbopol	70.2±10.8	80.5±13.6	76.1±12.9	63.4±9.1	32.1±6.4

The properties of the outer aqueous phase of the emulsion may also influence the diffusion of the drug from the inner to the outer water phase of the w/o/w emulsion during particle formation. Different drug loadings were measured for nanoparticles prepared with PVA, carbopol and poloxamer as stabilisers at similar pressures. The concentrations of the stabilisers were chosen as to give an equal viscosity to the water phases (1.45 mPa·s). But the stabilisers have different surface-active properties. Therefore, the differences of interfacial tension probably influenced drug diffusion from the water phase in the polymeric droplets to the outer water phase, resulting in different drug loadings for the different stabilisers employed. Apart from their surface-active properties, the electrical charges present on the stabilisers could also play a role. Spheres produced with carbopol showed higher drug loading compared to those produced with PVA or poloxamer. As was mentioned before, carbopol carries carboxyl groups which can be deprotonated leading to the formation of negatively charged groups. Pilocarpine HCl, as a salt, can be considered as a protonated, positively charged pilocarpine molecule. Possibly an electrostatic interaction between the carbopol and the pilocarpine can explain the higher encapsulation observed with carbopol.

The correlations between the drug encapsulation on the one hand and the particle size and the zeta potential value on the other hand are presented in Fig. 3 and in Fig. 4. In Fig. 3, the general trend is shown that larger particles have a higher drug encapsulation (correlation coefficient 0.6014). The higher forces employed during homogenisation lead to smaller emulsion droplets and consequently to smaller particle sizes. Simultaneously, an increased diffusion of pilocarpine HCl out of the forming particles results in a decreased drug encapsulation.

In Fig. 4 the correlation between the drug encapsulation and zeta potential values is presented (correlation coefficient −0.8090). The slope of the straight line can be mainly attributed to the difference between the data points for carbopol, having a high drug encapsulation and low zeta potential value on the one hand, and the data points for PVA and poloxamer having lower drug encapsulations and less pronounced zeta potential values on the other hand.

Drug Release The *in vitro* release studies were carried out using diffusion cells. The results of these experiments are presented in Table 5, Figs. 5 and 6.

When no homogenisation is applied about 70% of the drug is found in the receptor compartment after 24 h for particles stabilised with PVA. However, after homogenisation at 100 bar a complete release after 4 to 5 h is observed. It seems that the homogenisation results in a faster release. The drug release from particles prepared at 500 bar, 1 homogenisation cycle, shows a lag time, compared to the other preparations, while the particles prepared at three cycles show a somewhat faster release than the ones made without homogenisation. For poloxamer stabilised nanospheres, a release of 65% for non-homogenised particles was observed, which is quite similar to the result for the spheres prepared with PVA. An increased drug release rate after homogenisation at 100 bar is also seen. The results of the homogenisation at 500 bar are in

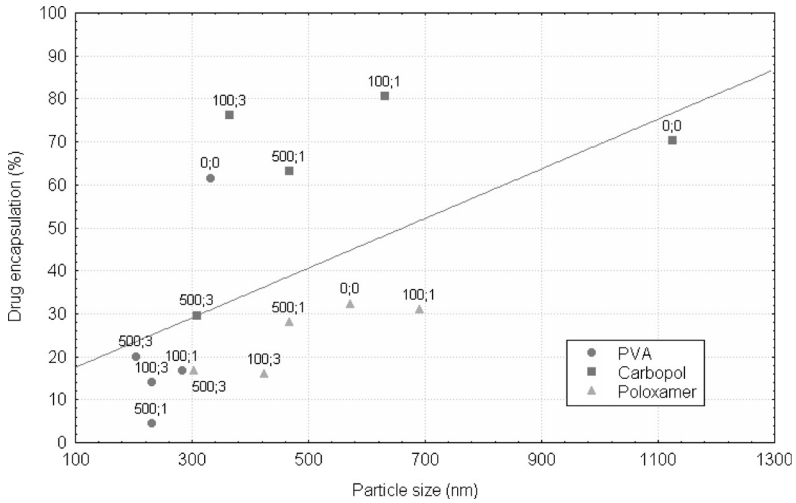

Fig. 3. Correlation between Particle Size and Drug Encapsulation of Pilocarpine HCl-Loaded PLGA Nanoparticles Prepared with PVA, Carbopol or Poloxamer as Stabiliser

At each data point the pressure applied during homogenisation and the number of cycles are printed.

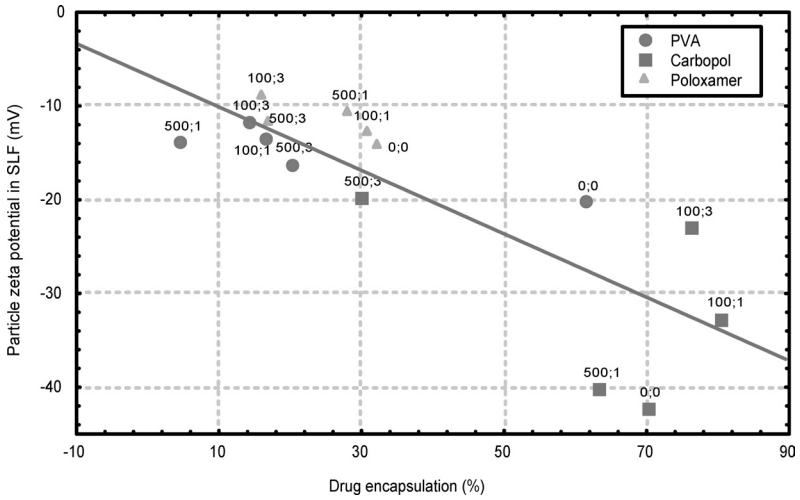

Fig. 4. Correlation between Drug Encapsulation and Zeta Potential Value of Pilocarpine HCl-Loaded PLGA Nanoparticles Prepared with PVA, Carbopol or Poloxamer as Stabiliser

At each data point the pressure applied during homogenisation and the number of cycles are printed.

Table 5. Total Pilocarpine Release (%) from PLGA Nanoparticles after 7 and 24 h

	Stabiliser									
	PVA		Poloxamer		Poloxamer+PVA		Carbopol		Carbopol+PVA	
Time (h)	7	24	7	24	7	24	7	24	7	24
0 ; 0	65	73	65	65	73	77	66	76	53	53
100 ; 1	100	100	84	99	100	100	100	100	69	93
100 ; 3	100	100	100	100	90	100	90	100	100	100
500 ; 1	26	43	68	81	71	80	74	98	71	91
500 ; 3	78	86	71	76	100	100	44	74	81	88

between those obtained with no homogenisation and the ones at 100 bar. With carbopol as a stabiliser, a release of 75% was measured for non-homogenised particles, a faster release for the homogenisation at 100 bar, and results in between both for homogenisation at 500 bar. The same trends were observed for the particles prepared with stabiliser mixtures.

The similarity of the release patterns between the particles stabilised with the different polymers suggest that the release does not depend strongly on the type of stabiliser. This seems quite surprising, since the type of stabiliser has an influence on all the other parameters investigated: particle size, particle zeta potential and drug loading.

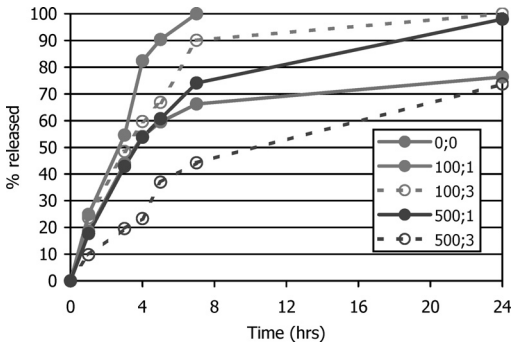

Fig. 5. Drug Release Profiles of Pilocarpine HCl-Loaded PLGA Nanoparticles Stabilised with Carbopol

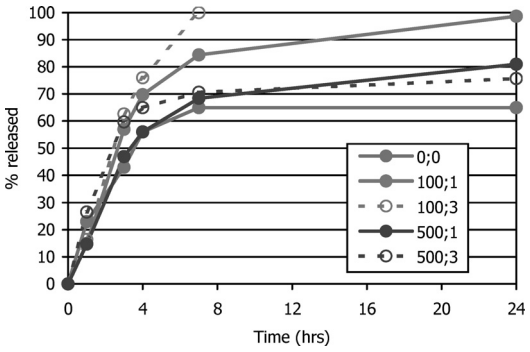

Fig. 6. Drug Release Profiles of Pilocarpine HCl-Loaded PLGA Nanoparticles Stabilised with Poloxamer

When the release patterns of the particles prepared without homogenisation and at a homogenisation pressure of 100 bar are compared, a faster release of the drug from the homogenised particles is observed. This can be explained by the decrease in particle size. Smaller particles have a higher total surface area, which should result in a higher burst release at the beginning of the diffusion tests. Moreover, if the release of the drug is controlled by diffusion out of the particle matrix, then smaller particles should also show a faster release, as the length of the pathway the drug has to follow to diffuse out of the particles is smaller.

Since particles prepared at 500 bar are even smaller than the ones prepared at 100 bar, a faster drug release was expected. The opposite is observed, so there should be another factor co-determining the release. Perhaps the lower drug encapsulation at higher homogenisation pressures becomes the determining factor. When lower drug encapsulations are obtained, the driving force for diffusion out of the matrix, the concentration gradient, becomes smaller, and consequently the release rate is decreased. On the other hand, Ueda and Kreuter have reported in their investigations with loperamide-loaded poly(L-lactide) nanoparticles that beside surface area of particles, their dense matrix structure could influence the drug release process.[11] In the present study, a difference between density structure of nanoparticles possibly occurred taking in account the different values of homogenisation pressure applied. This explanation could be deduced from the comparison between drug release profiles of two particle preparations with similar size. PLGA particles stabilised by PVA at a pressure of 100 bar, 3 cycles and prepared at 500 bar, 1 cycle have similar sizes, 232 nm and 231 nm, respectively. A faster drug release was observed for the particles prepared at the lower pressure. This would suggest that the pressure applied during homogenisation co-determines the release profile of the drug.

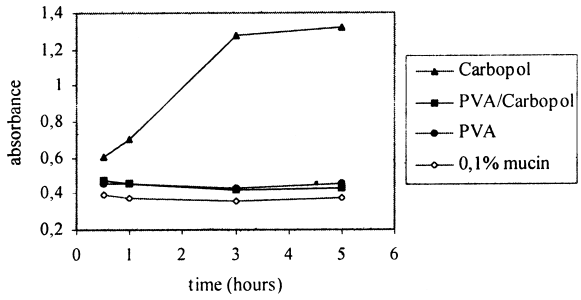

Fig. 7. Estimation of the Nanoparticle–Mucin Interaction by Turbidimetric Assay of Their Dispersions in Water; Nanoparticle/Mucin Ratio 1 : 1

Each point represents the value of triplicate runs.

Turbidimetric Measurements Turbidity of nanoparticle/mucin dispersions was examined in order to obtain preliminary information about the interaction of mucin and the PLGA particles. Two different ratios between nanoparticles and mucin in the aqueous dispersions were investigated. The absorbance of a native 0.1% mucin dispersion at 650 nm was used as a reference. Additionally, the optical density of mucin-free dispersions of nanoparticles in water was measured under the same conditions in order to see if their motion would influence the turbidity of the dispersions. The optical densities of the mucin-free dispersions containing the three kinds of nanoparticles did not significantly deviate from zero. Therefore, changes in the turbidity of nanoparticle/mucin dispersions should be attributed to the formation of an interaction product between nanoparticles and mucin. In fact, there was no remarkable difference in the turbidity of the dispersions containing PVA or PVA/Carbopol formulated PLGA-nanoparticles compared to the turbidity of the mucin dispersion itself (Fig. 7). Surprisingly, the profiles of both nanoparticle/mucin dispersions were practically identical to each other. There are some reports concerning the poor mucoadhesive properties of PVA[26,27] and an interaction between mucin and PVA layer was not expected. However, it was assumed that the addition of Carbopol to PVA in the mixed phase would provide eventually an interaction of PVA/Carbopol formulated nanoparticles with mucin. The data showed no significant difference between the turbidity of their dispersion compared neither to PVA nanoparticle/mucin nor to the mucin dispersion itself. The main reason was probably the formation of hydrogen bonds between carbopol and PVA during the preparation of the nanoparticles. Because of this interaction the Carbopol functional groups were already blocked and consequently not available to provide any interaction with the mucin. Additionally, the reduced number of carboxylate ions in the PVA/Carbopol layer was not sufficient to contribute to the hydration and diffusion processes, which participate in the mucoadhesion phenomena.

In contrast, the turbidity of the dispersion of nanoparticles formulated only with Carbopol was always much higher than that of mucin dispersion itself suggesting the formation of an interaction product.

The turbidity increased with time and was high even after five hours incubation independently on the nanoparti-

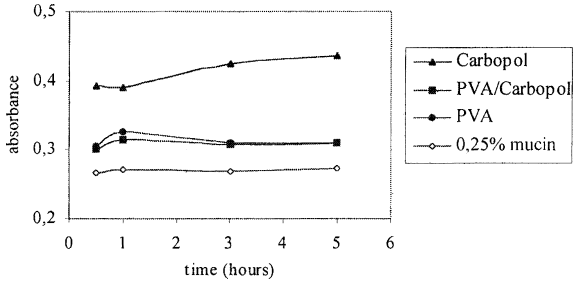

Fig. 8. Estimation of the Nanoparticle–Mucin Interaction by Turbidimetric Assay of Their Dispersions in SLF; Nanoparticle/Mucin Ratio 1 : 1

Each point represents the value of triplicate runs.

cle/mucin ratio. Hence, the presence of Carbopol into the outer water phase during the evaporation process lead to the formation of a coating layer around the particles disposing free Carbopol functional groups. Theoretically, many various mechanisms like physical entanglement, ionic interaction and hydrogen bonding contribute to mucoadhesion processes.[27,28] Regarding the present investigations the presence of a Carbopol layer around the particles enabled these processes especially the entanglement and hydrogen bonding with the mucin. The availability of more flexible Carbopol chains compared to the PVA/Carbopol mixed layer promoted the interpenetration which is the first stage of mucoadhesion phenomena. Further, because of the intimate contact the functional carboxyl groups of Carbopol interacted with the mucin functional groups forming hydrogen bonds. Similar observations were detected by rheological studies of viscous Carbopol/mucin dispersions.[22] Since the ion composition of lacrimal fluid may influence the interaction process the turbidity of nanoparticle/mucin dispersion in SLF was also examined. The results showed that the strongest interaction occurred between Carbopol formulated particles and mucin although it was less pronounced than the same interaction in water (Fig. 8). The weaker interaction in SLF could be explained by the presence of electrolytes in mucin/SLF medium compared to mucin/water one. It has been investigated that the Carbopol functional groups may react either with divalent (Ca^{2+}, Mg^{2+}) or with monovalent cations (Na^{+}) resulting in a compensation of their negative charge.[29] The lower anionic potential of Carbopol groups decreased the hydration of the absorbed surface layer which was the prerequisite for mucoadhesive interaction. These results were in agreement with Park and Robinson[30] who reported that the addition of NaCI caused reduction of the swelling degree of Carbopol. Taking in account both *in vitro* examinations in water and in SLF-medium it could be concluded that the Carbopol layer around nanoparticles disposed them to interact strongly with the mucin, promising a longer precorneal residence time due to mucoadhesion.

Conclusions

The present study shows that in the preparation of PLGA nanoparticles, the homogenisation process as well as the choice of stabilizers can be used to adapt the particle properties. Not only the size of the spheres, but also their zeta potential value, drug loading and drug release depend on the homogenisation pressure and number of cycles. The study shows that if a small particle size is desired, a higher homogenisation pressure, combined with a higher number of cycles should be applied. The use of carbopol as a stabiliser was shown to have several advantages: a negative effect on the drug loading can be avoided, resulting in small particles with a high drug loading. Moreover, the preliminary data of the turbidimetic data also shows that carbopol-coated nanoparticles could show mucoadhesive properties.

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